18F-fluorodeoxy-glucose positron emission tomography (18FDG-PET) marks MYC-overexpressing human basal-like breast cancers

Nicolaos Palaskas 1,4, Steven M. Larson 7, Nikolaus Schultz 12, Evangelia Komisopoulou 1,4, Justin Wong 1,4, Dan Rohle 5, Carl Campos 13, Nicolas Yannuzzi 13, Joseph R. Osborne 6, Irina Linkov 8, Edward R. Kastenhuber 8, Richard Taschereau 1,4, Seema B. Plaisier 1,4, Chris Tran 13, Adriana Heguy 13, Hong Wu 2,3,4, Chris Sander 12, Michael E. Phelps 1,2,3,4, Cameron Brennan 9,13, Elisa Port 10, Jason T. Huse 8,13, Thomas G. Graeber 1,2,3,4,^, and Ingo K. Mellinghoff 5,11,13,^  

1 Crump Institute for Molecular Imaging, 2 Institute for Molecular Medicine, 3 Jonsson Comprehensive Cancer Center, 4 Department of Molecular & Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles CA 90095, USA; Department of 5 Pharmacology and Radiology, Weill-Cornell Medical College, New York, NY 10065, USA; 7 Departments of Nuclear Medicine, 8 Pathology, 9 Neurosurgery, 10 Surgery, and 11 Neurology; 12 Computational Biology Center; and 13 Human Oncology & Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY10021. ^ equal contribution.

CORRESPONDING AUTHORS: T. Graeber, University of California Los Angeles, 570 Westwood Plaza, CNSI 4341, Los Angeles, CA 90095. E-mail: tgraebert@mednet.ucla.edu and I. Mellinghoff, Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Mailbox 20, New York, NY 10021. E-mail: mellingi@mskcc.org
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RUNNING TITLE: Genomic Determinants of $^{18}$F-fluorodeoxy-glucose-uptake in cancer

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ABSTRACT

In contrast to normal cells, cancer cells avidly take up glucose and metabolize it to lactate even when oxygen is abundant, a phenomenon referred to as the Warburg effect. This fundamental alteration in glucose metabolism in cancer cells enables their specific detection by Positron Emission Tomography (PET) following intravenous injection of the glucose analogue 18F-fluorodeoxy-glucose (18FDG). However, this useful imaging technique is limited by the fact that not all cancers avidly take up FDG. To identify molecular determinants of 18FDG-retention, we interrogated the transcriptomes of human cancer cell lines and primary tumors for metabolic pathways associated with 18FDG radiotracer uptake. From 95 metabolic pathways that were interrogated, the glycolysis and several glycolysis-related pathways (pentose-phosphate, carbon fixation, aminoacyl-tRNA biosynthesis, one-carbon-pool by folate) showed the greatest transcriptional enrichment. This “FDG signature” predicted FDG-uptake in breast cancer cell lines and overlapped with established gene expression signatures for the “basal-like” breast cancer subtype and MYC-induced tumorigenesis in mice. Human breast cancers with nuclear MYC staining and high RNA expression of MYC target genes showed high 18FDG-PET uptake (p < 0.005). Presence of the FDG signature was similarly associated with MYC gene copy gain, increased MYC transcript levels, and elevated expression of metabolic MYC target genes in a human breast cancer genomic dataset. Together, our findings link clinical observations of glucose uptake with a pathologic and molecular subtype of human breast cancer. Further, they suggest related approaches to derive molecular determinants of radiotracer retention for other PET-imaging probes.
INTRODUCTION

Glycolysis and oxidative phosphorylation represent the main metabolic pathways that fuel energy dependent processes in cells by generating adenosine triphosphate (ATP). Compared to normal differentiated cells, cancer cells show increased glycolytic rates and high lactate production in-vitro even when oxygen levels are sufficient to support oxidative phosphorylation, a process called aerobic glycolysis or “Warburg effect” (1). Why cancer cells favor an energetically less efficient path of glucose utilization and how they avoid oxidative phosphorylation has remained unclear for decades. A number of recent observations have begun to shed light on the latter question. Knockdown of LDH-A in *neu*-initiated mammary epithelial tumor cell lines stimulated mitochondrial respiration, indicating that the glycolytic phenotype of cancer cells is not necessarily due to intrinsic mitochondrial defects in oxidative phosphorylation. Furthermore, activation of tyrosine kinase signaling, frequently caused by mutations in growth factor signaling pathways, has been found to inhibit the entry of pyruvate into the mitochondrial TCA cycle through hypoxia-inducible factor 1 (HIF1) mediated induction of pyruvate dehydrogenase kinase 1 (PDK1) and posttranslational modification of the M2 splice isoform of pyruvate kinase (2).

The altered glucose metabolism of tumor cells can be observed in cancer patients by positron emission tomography (PET) following intravenous injection of the glucose analogue $^{18}$F-fluorodeoxyglucose ($^{18}$FDG)(3). Compared to normal surrounding tissue, tumors often show an increase in the FDG-PET signal which reflects their high rate of radiotracer uptake through membrane glucose transporters, phosphorylation by one of several hexokinase enzymes, and the resultant intracellular trapping of the radiotracer which is not further metabolized in the cell. Not all cancers, however, avidly take up FDG. Human breast cancers, for example, show up to 20-fold differences in their FDG-PET signal. This heterogeneity has been attributed, with substantial variation between studies, to differences in histopathologic subtypes, tumor size, microvasculature, tumor cell proliferation, hormone receptor status, and expression levels of hexokinase or glucose transporters (4).
Despite the widespread use of FDG-PET imaging in oncology, the relationship between FDG uptake of primary human tumors and their metabolic and genetic alterations is largely unknown. To address this question, we performed genome wide transcriptomal analysis of cell lines and primary human tumors after determining their FDG uptake. We found that $^{18}$FDG retention is associated with coordinated transcriptional upregulation of multiple metabolic pathways, including the core glycolysis, pentose-phosphate, and carbon fixation pathways. This “FDG signature” predicted radiotracer uptake in breast cancer cell lines and was closely linked to the “basal-like” intrinsic breast cancer subtype and activation of the MYC oncogene.

**MATERIALS AND METHODS**

**Cell lines.** LAPC4 and HCT116 PTEN knockout cells were derived and provided by Dr. Sawyers and Dr. Waldmann, respectively. All other cell lines were obtained from ATCC and the NCI.

**Patients.** Eighteen breast cancer patients who presented for operative management of primary breast carcinoma were imaged with FDG-PET within 4 weeks prior to surgery; excluding patients with secondary breast cancers and recurrent disease. None of the patients received systemic therapy or radiation prior to imaging. All breast tumor samples were collected surgically. Our study included one patient with anaplastic astrocytoma. This study was approved by the IRB of Memorial Sloan-Kettering Cancer Center and all participating patients signed informed consent.

**Gene expression analysis.** Gene expression signature-based predictions of FDG uptake were made using weighted gene voting (WGV)(5). The “rank-rank hypergeometric overlap” (RRHO) algorithm (6) was used to examine the statistical significance of similarity between our FDG signature and other gene expression signatures. Details of the bioinformatic approaches are described under Supplementary Methods.
RESULTS

Upregulation of glycolysis and glycolysis branch pathways in 18FDG-avid cancer cells.

Our strategy to identify determinants of FDG-retention consisted of measurements of 18FDG retention in cancer cell lines and primary human tumors, RNA expression profiling of these samples, comparison of 18FDG “high” versus “low” samples using gene set enrichment analysis (GSEA) (7), and mining of genomic datasets for this “FDG signature” (Fig.1A).

We first measured 18FDG radiotracer uptake of sixteen cancer cell lines representing prostate cancer (CaP), glioblastoma (GBM), and melanoma (MEL). We observed up to 5-fold differences in FDG-uptake between cell lines of the same histologic type (Fig.1B). The clinical sample set included tumors from eighteen breast cancer patients. 18FDG-tumor uptake was quantified as standardized uptake values (SUVs) and showed the expected wide dynamic range (0 to 22.1)(Fig.1C, top). Breast cancers with the highest 18FDG-PET SUVs frequently lacked expression of the estrogen receptor (ER) and the progesterone receptor (PR), but hormone receptor negative tumors were also represented amongst the tumors with the lowest FDG-uptake (Table 1). Our clinical sample set also included a patient with anaplastic astrocytoma whose tumor showed areas of distinct FDG-uptake. Both an FDG-high and FDG-low region were amenable to stereotactic biopsy (Fig.1C, bottom) and represented viable tumor.

For each tumor type represented in our panel, we selected samples with particularly high and low FDG-uptake for gene set enrichment analysis (GSEA) (7) using 95 metabolic pathways annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG)(8). We hypothesized that a comparison of samples at the extremes of the FDG-uptake spectrum would facilitate the identification of FDG-uptake associated metabolic pathways. The following FDG-high versus FDG-low sample sets were used: i.) LNCaP vs. LAPC4 cells (prostate cancer), ii.) U87 vs. SF268 (glioblastoma), and iii.) SKMel28 vs. CHL cells (melanoma)(marked with asterisk in Fig.1B), iv.) FDG-high vs. FDG-low region of the anaplastic astrocytoma, and v.) breast cancers with SUVs above 10 vs. breast cancers with SUVs below 5 (marked with asterisk in Table 1). We initially excluded lobular breast
carcinomas, because they have been shown to take up less FDG than ductal carcinomas (9). We initially also excluded large breast carcinomas (>5 cm) and breast carcinomas with multifocal FDG-uptake because our protocol did not include tissue autoradiography to direct the molecular tissue analysis to areas of distinct radiotracer retention. There was no significant difference in patient age, tumor volume, and lymph node involvement between the group of FDG-high and FDG low breast cancers.

For our combined GSEA analysis using the 5 FDG-high vs. FDG-low sample sets and the 95 KEGG metabolic pathways, we first defined a rank-based gene expression signature for each histology type (breast and astrocytoma tumors; and prostate, glioblastoma and melanoma cell lines). Then the average rank for each gene was determined to define an average-rank signature that was interrogated using GSEA. The glycolysis/gluconeogenesis pathway scored as the most highly enriched metabolic pathway. The related carbon fixation and pentose-phosphate pathways also showed significant enrichment in the FDG-high samples, as well as the pathways for aminoacyl-tRNA biosynthesis and one-carbon-pool by folate (Fig.1D). Results were consistent between this average-rank approach and enrichment analysis of the individual signatures (Table S1).

To exclude the possibility that our selection of breast cancer samples had introduced experimental bias, we repeated the GSEA analysis with all 18 primary breast cancer samples using a continuum SUV correlation-based ranking approach. Enrichment of the glycolysis, pentose phosphate, and carbon fixation remained significantly associated with the $^{18}$FDG-PET signal (Table S1, Column “Breast Cancer, SUV Continuum).

**Gene-expression based “FDG signature” predicts FDG-uptake in-vitro.**

We next examined whether pathway enrichment was driven by modest differences in the levels of many pathway members or more dramatic effects on only 1-2 key enzymes. As illustrated in Fig. 2A for the glycolysis core pathway in the breast cancer samples, pathway enrichment was due to moderate (less than 2-fold), but highly concordant differences in the transcript levels of many
pathway members. Similarly modest differences in transcript levels of functionally related genes have been shown to regulate metabolic flow in other biological and disease systems (10-12).

We also determined the contribution of individual enzymes to overall pathway enrichment based on their rank in each separate GSEA analysis (Fig.2B and Fig.S1). While there were differences between tissue types (Fig.S1B), enzymes that direct metabolic flow towards glycolysis (e.g., phosphofructokinase, hexokinase, pyruvate kinase) showed the most consistent enrichment within the glycolysis/glucoseogenesis KEGG pathway (Fig.2B and Fig.S1B-C). The mean rank in the combined analysis for members of the top three enriched metabolic pathways (glycolysis, pentose-phosphate, carbon fixation) is shown in Figure 2B. The “carbon fixation” KEGG pathway is functionally complete only in plants and scored in our analysis due to the overlap of its enzymes with the glycolysis/glucoseogenesis and pentose-phosphate pathways.

We next tested whether transcript levels of the most highly ranked members of the top three enriched metabolic pathways, could serve as an “FDG signature” and predict FDG-uptake. We explored this question in a panel of seven human breast cancer cell lines which were not included in our initial FDG-uptake studies. We selected breast cancer cell lines for the validation of our FDG signature because the majority of human tumor samples used for the derivation of this signature were breast carcinomas. Predictions were made using the weighted gene voting approach (13) and using the primary breast tumors as the FDG signature training set (Table S2, Fig. S1C). FDG-uptake assays were performed blinded to our computational analysis and showed a wide range of FDG-uptake, as has been reported for breast cancer cell lines (14). We found a strong correlation between measured FDG-uptake and predicted FDG-uptake ($r = 0.92$, permutation $p$ value $= 0.03$)(Fig.2C).

We next tested weighted gene voting predictions using individual genes with the greatest differential expression between FDG high and FDG low breast cancers (top 100 – top 2000 genes). Predictions of FDG uptake using the top differentially expressed individual genes showed less correlation with the measured FDG-uptake (Fig.2D), suggesting that our metabolism-oriented
bioinformatic approach uncovered a shared metabolic state in FDG high samples that would be more
difficult to detect using “gene-centric” data analysis approaches.

**FDG signature is associated with the “basal-like” breast cancer subtype.**

The derivation of a gene expression-based FDG signature enabled us to search published
genomic datasets for the presence of this signature with the goal to identify tumor types, genetic
lesions, or signaling pathways that might be associated with FDG uptake. We focused this analysis
on human breast cancer because of the wealth of validated RNA expression signatures in this
disease (15). We first developed a method for quantitating the degree of overlap between two
signatures defined by differential gene expression (6)(Fig. S2). We then applied this “rank-rank
hypergeometric overlap” (RRHO) method to a dataset of 295 primary human breast cancers (16) to
determine the overlap between the FDG signature and the main intrinsic breast cancer subtypes, i.e.
“basal-like”, “luminal”, “HER2/ ErbB2”, and “normal-like”. We found significant overlap with the
signature for the “basal-like” subtype, an inverse relationship with the signature for the luminal and
normal subtypes (all with p-values < 10^{-4}), and no overlap with the ErbB2 subtype (Fig.3A).

Our results indicated that the expression of genes in basal-like breast cancers resembled
FDG-high cancer cells more closely than any other breast cancer subtype. If true, a direct GSEA
comparison between “basal-like” and “other” breast cancers should identify similar metabolic
pathways as our previous GSEA comparison between “FDG-PET high versus FDG-PET low” breast
cancers. We tested this hypothesis in a second dataset of 286 primary human breast cancers (17).
Only two metabolic pathways were significantly enriched in this analysis (glycolysis/gluconeogenesis
and aminoacyl-tRNA biosynthesis)(Table S3), both of which had demonstrated strong enrichment in
our prior GSEA comparison of FDG high versus FDG low human primary breast cancers (Table S1).

We further tested whether our “FDG signature score”, which had predicted FDG-uptake in
breast cancer cell lines (Fig.2C), would preferentially identify basal-like breast cancers within a
sample set representing all breast cancer subtypes. To test this hypothesis, we used metabolic
pathway-based weighted gene voting to predict the FDG-uptake of tumors in a third independent dataset of 80 locally advanced primary human breast cancers (18). Consistent with our prior analysis, 14/18 (77.8%) tumors with the highest FDG-signature score were of the “basal-like” subtype, compared to 0/18 (0%) tumors with negative FDG signature score (multivariate hypergeometric p-value, $10^{-8}$). A Kolmogorov-Smirnov sliding threshold-based analysis using all 80 tumors also yielded statistical significance with a permutation p-value of $10^{-7}$ (Fig.3B)(Table S4).

Basal-like breast cancer has been shown to harbor a greater number of low level gene copy number alterations than other breast cancer subtypes (19) and breast carcinomas with highest FDG-signature score harbored a significantly greater number of gene copy number alterations than tumors lacking the FDG signature (Fig.3C, top). Based on these results, we performed array-cGH profiling of breast cancer samples for which we had residual frozen tissue (11 of the original 18). We found significantly more gene copy number alterations in the group of FDG-high tumors (SUV>10) compared to the group of FDG low tumors (SUV<5)(Fig. 3C, bottom), providing further evidence that FDG-avid breast carcinomas exhibit genetic properties of the “basal-like” breast cancer subtype.

**Activation of MYC in FDG-avid breast cancers.**

Mutant alleles of ras and activation of the phosphatidylinositol 3-kinase (PI3-K) pathway have been shown to increase glucose uptake in experimental models (20). None of the breast tumors in our collection harbored an activating mutation in a ras-family member (*data not shown*). We found activating mutations in the catalytic subunit of PI(3)K in three breast carcinomas (BT07: C420R; BT09: H1047R; BT04: H1047R), all three estrogen receptor positive tumors, consistent with the reported association between PIK3CA mutations and ER-positivity in breast cancer (21). We next mined our breast carcinomas for transcriptional evidence of increased PI(3)K pathway activity, based on similarity with signatures derived from an Akt-driven murine tumor model (22) and human breast cancer samples lacking expression of the PTEN tumor suppressor (23). As with our FDG uptake signature (Fig. 1D), these PI(3)K-activation signatures showed enrichment of the glycolysis, pentose
phosphate and carbon fixation pathways (Fig. S3A), and this enrichment was driven by similar glycolysis promoting enzymes (Fig. S3B).

We next examined protein levels of PTEN by immunohistochemistry (Fig. S3C) as diminished protein levels of this tumor suppressor have been reported for 15-25 % of all breast carcinomas and more commonly in basal-like breast cancer (24). Compared with adjacent non-neoplastic cells, we observed reduced PTEN staining of tumor cells in 6/16 (37.5 %) of tumors; five of the PTEN-deficient breast carcinomas were in the group of tumors with highest FDG-PET uptake. We next examined the effects of PTEN inactivation on cellular FDG-uptake in cancer cell lines. In HCT116 colon cancer cells (25), which harbor mutations in PIK3CA and KRAS, PTEN knockout raised the FDG uptake by about 50% (p<0.05) (Fig.S3D). PTEN knockdown in three other cancer cell lines (A431, HCC827, SKBR3), on the other hand, only raised FDG-uptake in one of the lines (SKBR3) and this increase was not statistically significant (Fig.S3E). These results suggest that the effects of PTEN on glucose metabolism are cell context-specific.

To identify additional signaling pathways that are associated with FDG-PET uptake in breast cancer, we searched a Molecular Signatures Database (MSigDB) for our FDG signature. This database is comprised of 1822 gene sets representing canonical signaling pathways, cellular processes, chemical and genetic perturbations, and human disease states (7). 110 of the 1822 gene sets, extracted directly from MSigDB without modifying their contents, were positively enriched in the transcriptome of FDG-high samples (Table S5). The top gene sets included gene sets related to poor prognosis (rank 6, 9) and high tumor grade (rank 25) in breast carcinoma. The top gene sets also included multiple gene sets both directly and indirectly related to the transcription factor MYC (Fig. 4A)(Table S5). The direct MYC group was comprised of gene signatures that are upregulated in transgenic mouse models of c-myc induced cancer (rank 12, 28, 49) (26-28); MYC-related gene signatures included the serum fibroblast response/ wound healing signature (rank 2, 17, 39) linked to MYC activation in breast cancer (29-31), and a signature linked to MYC activation in lymphoma (rank 38)(32). We found an inverse relationship (i.e., negative enrichment score) between our FDG
signature and genes repressed by MYC (rank 1628, 1643, 1751) (26, 33, 34). The overall association between our FDG signature and gene sets directly linked to MYC was statistically significant \((p=0.002)\) (Fig. 4A).

We next stained all breast carcinomas for which we had remaining tissue (16/18) with an antibody against the MYC protein. 8/16 (50 %) tumors showed nuclear staining of tumor cells, similar to the reported frequency of MYC immunoreactivity (40-50 %) in human breast cancer (35, 36) (Fig. 4B, left). MYC immunopositivity was significantly associated with high \(^{18}\)FDG PET SUV-values \((p=0.002)\) (Fig. 4B, right). Nuclear localization of MYC was associated with increased MYC transcriptional activity based on the overexpression of genes under direct transcriptional control of MYC in the MYC IHC-positive group, including the glutamine transporter SCL7A5 (37) \((p<0.001)\), serine hydroxymethyl-transferase (SHMT) (38) \((p<0.05)\), lactate dehydrogenase LDH-A (39) \((p < 0.05)\), and transferrin receptor 1 (TFRC1) (40) \((p<0.01)\) (Fig. 4C).

We also examined the relationship between the FDG-signature score, MYC levels and MYC target gene expression in the breast cancer dataset (18) used in our prior analysis (Fig. 3B). The frequency of \(c\)-myc copy gain \((\log_2 \text{ratio} \geq 0.4)\) in the top half of the FDG-signature score ranked tumors \((19/40= 47.5\%)\) significantly exceeded the frequency of \(c\)-myc copy gain in the bottom half \((6/40 = 15 \%)\) and in the entire cohort of patients \((25/80= 31.3 \%)(\text{hypergeometric } p-value=0.002)\) (Fig. 4D), demonstrating that the FDG signature significantly enriches for tumors harboring this molecular alteration. When we focused on the subgroup of tumors with the highest versus lowest FDG signature scores, we found elevated \(c\)-myc gene dosage in 10/18 (55.5 \%) of breast cancers with high FDG signature score, but none \((0/18)\) of the breast cancers with low FDG signature score \((t\text{-test } p=0.0002)\). MYC transcript levels and the expression of MYC target genes were similarly statistically associated with the FDG signature score (Fig. 4D).

As genes induced by hypoxia also emerged from our pathway analysis (rank 8, 79) (Fig. 4A), we also stained all breast tumors with an antibody against hypoxia-induced factor (HIF-1). HIF1α is overexpressed in human cancers as a result of intratumoral hypoxia and genetic alterations in tumor
cells (41). 12/16 (75%) tumors showed intense nuclear staining for HIF1α (Fig.S4A), including all breast carcinomas with nuclear MYC-staining and highest FDG-PET signal. 7/12 HIF-1 positive tumors, but none of the HIF-1 negative tumors, also showed cytoplasmic staining with an antibody against phosphorylated proline-rich AKT1 substrate 1 (PRAS40)(Fig.S4B/C), a target of Akt and readout for PI(3)K pathway activity (42).

**DISCUSSION**

FDG-PET is widely used in the clinic for the detection of cancer. Despite a wealth of data linking glucose uptake to mutations in oncogenes and tumor suppressor genes in vitro (20), most studies of primary human tumors have focused on expression levels of hexokinase and glucose transporters. Our study sought to define the broader context of metabolic and genetic alterations in FDG-avid cancers. We show that FDG-avid tumors share a transcriptional program that involves not only members of the core glycolysis pathway, but also several glycolysis branch pathways critical for nucleotide and amino acid synthesis. These findings support the model that cancer cells favor aerobic glycolysis, despite the “penalty” of inefficient ATP production, because its metabolic intermediates can be used by the proliferating cancer cell for the replenishment of NADPH and the synthesis of highly needed macromolecules (43).

We identified overexpression of the transcription factor MYC as the molecular alteration most highly associated with FDG-uptake in human breast cancer. MYC is a plausible candidate to orchestrate the metabolic program of FDG-avid cancers. NMR studies have shown that MYC regulates the flux of glucose carbon not only through the core glycolysis pathway, but also through glycolysis branch pathways which were consistently upregulated in our analysis (i.e., pentose-phosphate pathway, amino-acid metabolism, and C1/folate metabolism)(44). Furthermore, MYC directly regulates RNA levels of several members of the glycolysis and glutamine pathway which showed increased transcript levels in FDG-avid (Fig. 4C) and FDG-signature positive tumors (Fig. 4D). These include PDK1 and LDH-A, which attenuate entry of pyruvate into the TCA cycle, the
glutamine transporters SLC7A5 and SLC1A5, and – with some differences between published experimental models (45, 46) – glutaminase (GLS).

FDG-uptake in breast cancer did not correlate with Akt activation, a finding previously reported in short-term human breast cancer cultures (14). Our further examination of PI(3)K “pathway output” showed that the PI(3)K pathway is nonetheless activated in the majority of FDG-avid breast cancers, perhaps through alterations parallel or downstream of Akt (47). In peripheral nerve sheath tumors induced by monoallelic PTEN inactivation and mutant K-ras in mice, loss of the second PTEN allele coincides with a marked increase in tumor FDG-uptake (48), suggesting that the strength of PI(3)K pathway activation may be an important determinant of the glycolytic state. PI(3)K pathway activation may also cooperate with other oncogenic events, such as MYC, to induce a maximally glycolytic state. Several genes which were significantly upregulated in FDG-avid breast cancers in our study have previously been shown to be regulated by both MYC and HIF-1, including LDH-A, PDK1, and transferrin receptor I. Studies in a larger panel of primary human tumors are warranted to define the relationship between FDG-uptake, the PI(3)K-HIF1 axis, and other cancer genes.

The role of FDG-PET imaging in the management of human breast cancer remains to be defined (49). One of the challenges is the detection of small tumors (< 2.0 cm) as partial volume effects (PVEs) can result in underestimation of true radiotracer retention (50). This may have affected our estimation of the SUVs and should be addressed in future studies using different PVE correction schemes (51). Our study connects the clinical observation of altered glucose metabolism with a molecular subtype of human breast cancer, namely basal-like breast cancer with MYC activation. This conclusion, reached through a genome-wide approach, links prior observations that i.) the basal-like breast cancer subtype is enriched for tumors with MYC copy gain (52) and a MYC gene expression signature (30, 31) and that ii.) breast cancers which lack expression of estrogen receptors, progesterone receptors, and HER2 gene amplification (“triple-negative”), as is true for the majority of basal-like breast cancers, have shown increased FDG-uptake in larger clinical studies.
(53) (54). However, basal-like breast cancers are defined by their gene expression profile, express ER or overexpress HER2 in up to 20 % of cases, and represent a disease subgroup that is distinct from triple-negative breast cancer (55). Our findings suggest that FDG-PET may be particularly useful as biomarker for therapies that target the basal-like breast cancer subtype or the “addiction” of MYC-induced tumors to the glycolysis and glutamine pathway (45, 46).

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REFERENCES


FIGURE LEGENDS

Figure 1. Deriving an “FDG-uptake” metabolic gene expression signature. (A) Schematic of our experimental approach. (B) 18F-FDG retention in 16 cancer cell lines, including prostate cancer (CaP), glioblastoma (GBM), and melanoma (MEL). Error bars indicate standard error. (C) Clinical datasets. (Left) Tumor FDG-uptake in 18 breast cancer patients. SUV, Standardized Uptake Value. (Right) FDG-PET scan and Brain Magnetic Resonance Imaging (MRI) in a patient with anaplastic astrocytoma. (Area 1 FDG-high, Area 2 FDG-low). Cross-hairs are centered at sites of biopsy. (D) ‘High FDG uptake’ samples show transcriptional enrichment for glycolysis and glycolysis-branch metabolic pathways. Shown are average rank-based GSEA results (see Table S1 for the results of all KEGG metabolic pathways).

Figure 2. FDG signature score predicts in-vitro FDG-uptake. (A) RNA levels of core glycolysis enzymes (red) versus all genes (gray) in high FDG-PET (n=5, y-axis) versus low FDG-PET (n=6, x-axis) breast carcinomas. Tumors included in the analysis are denoted with asterisks in Table 1. (B) Average rank of Glycolysis/Gluconeogenesis, Carbon Fixation, and Pentose Phosphate Pathway enzyme members across all sample sets (see also Fig. S1). Low rank numbers represent high expression in the “FDG high” samples. Enzymes in green promote glycolysis, while those in red promote gluconeogenesis. The core glycolysis pathway was included as a point of reference. Enzyme rankings for the primary breast tumor-based FDG signature alone are shown in Fig. S1C. The schematic below shows the glycolysis/ gluconeogenesis pathway (and related pathways) as annotated by KEGG. Enzyme names are in italics. (C) Correlation between the observed (x-axis) and predicted (y-axis) FDG-uptake (see text and Fig, S1C for details) in seven breast cancer cell lines. The correlation (r=0.92) was statistically significant (sample label permutation p-value = 0.03). Cell lines from low to high FDG uptake are: HCC1500, BT474, ZR7530, ZR751, HCC70, UACC812, MCF7. (D) Correlations between observed FDG uptake and ‘FDG signature score’ predictions, using either only genes from the three glycolysis-related metabolic pathways which comprise the “FDG
signature” (as in panel C; gly+cf+pp) or ‘lists of the top n genes’ with the highest differential expression between FDG-high and FDG-low breast cancers. Error bars: standard error.

**Figure 3.** FDG signature overlaps with “basal-like” breast cancer subtype. (A) Overlap between the FDG uptake signature and signatures for intrinsic breast cancer subtypes (16). Using the rank-rank hypergeometric overlap (RRHO) approach (Fig. S2), genes were ranked by their degree of correlation with FDG-PET SUV values across the tumors (n=18) or their degree of differential expression between the indicated subclasses to define the rank-based signature. The Spearman rank correlation coefficient (\(\rho\)) between signatures was calculated. All cases, except the ERBB2 case, had significant correlation based on sample permutation-based statistical analysis (p-value < 0.0001). (B) FDG-signature score preferentially identifies basal-like breast tumors. (Left) Schematic of experimental approach. (Right) Rank-ordered distribution of intrinsic human breast cancer subtypes relative to their predicted FDG signature score. See also Table S4. (C) Elevated genomic instability of human breast cancers with high ‘FDG signature score’ (top, n=18 high, 18 low) or high FDG-PET uptake (bottom, FDG-PET SUV>10 [n=5] compared to SUV < 5 [n=5]). Shown are cGH profiles with regions of copy-number gain (loss) shown as shades of red (blue). The graphs on the right show a quantification of gene copy number alterations. The higher absolute number of transitions per chromosome in the lower plot (versus upper plot) is due to the higher resolution of the cGH platform.

**Figure 4.** MYC activation in FDG-high primary human breast cancers. (A) Rank of MSigDB gene sets when analyzed for enrichment in the FDG-uptake signature. NES: normalized enrichment score. Enrichment results for all 1822 MSigDB C2 gene sets are listed in Table S5. (B) MYC IHC-positive breast cancers have higher FDG-PET SUV values. (Left) Representative MYC-IHC images (40x). Sample numbers and FDG-PET SUVs (in parenthesis) are shown above each panel. (Right) Distribution of FDG-PET SUVs in MYC IHC positive versus negative breast carcinomas. (C) RNA
levels of MYC and MYC target genes in tumors with negative (left) versus positive (right) nuclear MYC staining by IHC. Red = high; p-values represent t-test analysis of the MYC IHC negative vs positive samples. (D) Breast Cancers with high FDG signature score are more likely to have elevated c-myc gene dosage (MYC DNA), MYC transcript level (MYC RNA), and MYC target gene expression. Tumors are ordered by FDG signature score (low to high); p-values represent a t-test analysis of the top 40 versus bottom 40 scoring FDG signature samples. Similar p-values were obtained for top/bottom 18 samples, or for the correlation of copy number or expression values with the FDG signature score.
Table 1. FDG-PET tumor uptake in 18 patients with locally advanced breast cancer

<table>
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<tr>
<th>PATIENT ID</th>
<th>FDG-UPTAKE [SUV max]</th>
<th>AGE</th>
<th>SEX</th>
<th>TUMOR SIZE [cm]</th>
<th>HISTOLOGY</th>
<th>HISTOgrade</th>
<th>NUC GRADE</th>
<th>LYMPH NODE INVASION</th>
<th>ER</th>
<th>PR</th>
<th>HER2 DNA</th>
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<td>NEG</td>
<td>NEG</td>
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<tr>
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<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
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<td>29</td>
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<td>2.3</td>
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<td>III</td>
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<td>NEG</td>
<td>NEG</td>
<td>NOT AMP (1.0)</td>
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<td>NEG</td>
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<td>NOT AMP (1.6)</td>
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<td>III</td>
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<td>POS</td>
<td>POS</td>
<td>NOT AMP (1.0)</td>
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<tr>
<td>BT07 *</td>
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<td>69</td>
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<td>4.2</td>
<td>DUCTAL</td>
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<td>III</td>
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<td>POS</td>
<td>NOT AMP (1.2)</td>
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<td>DUCTAL</td>
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<td>II</td>
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<td>POS</td>
<td>NOT AMP (1.3)</td>
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<td>59</td>
<td>F</td>
<td>4.5</td>
<td>DUCTAL</td>
<td>III</td>
<td>III</td>
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<td>NEG</td>
<td>NEG</td>
<td>NOT AMP (1.0)</td>
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<tr>
<td>BT09 *</td>
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<td>62</td>
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<td>POS</td>
<td>NOT AMP (1.1)</td>
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<td>YES</td>
<td>POS</td>
<td>POS</td>
<td>NOT AMP (1.0)</td>
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<td>NEG</td>
<td>NEG</td>
<td>AMP (5.4)</td>
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<td>LOBULAR</td>
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<td>N/A</td>
<td>YES</td>
<td>POS</td>
<td>NEG</td>
<td>NOT AMP (1.0)</td>
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</tbody>
</table>

*samples included in the biphenotypic GSEA comparison of "FDG high"(SUV>10) versus "FDG low"(SUV<5) tumors. N/A not available. NEG negative. POS positive. AMP amplified.
A. Determine FDG uptake in cancer cell lines and primary human tumors.

B. Collect genome-wide RNA expression data.

C. Identify metabolic pathways (KEGG database) that are transcriptionally enriched in FDG high samples.

D. Discover relationship of FDG signature to:
   - other gene expression signatures (e.g., disease subtypes, mouse models of human cancers, signaling signatures)
   - DNA copy number alterations

D. Metabolic Pathway (KEGG) enriched in High FDG Uptake

<table>
<thead>
<tr>
<th>Metabolic Pathway (KEGG)</th>
<th>AVE RANK-BASED GSEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLYCOLYSIS / GLUCONEOGENESIS</td>
<td>NES</td>
</tr>
<tr>
<td>CARBON FIXATION</td>
<td>2.17</td>
</tr>
<tr>
<td>PENTOSE PHOSPHATE PATHWAY</td>
<td>1.94</td>
</tr>
<tr>
<td>AMINOACYL-TRNA BIOSYNTHESIS</td>
<td>1.88</td>
</tr>
<tr>
<td>ONE CARBON POOL BY FOLATE</td>
<td>1.88</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
Glycolysis core

Average rank in FDG-uptake gene list

PFKL PFKM PFKP 41
HK1 HK2 HK3 76
GPI 95
PKLR PKM2 111
PGK1 PGK2 314
ALDOA ALDOB ALDOC 523
ALDH1A3/3A1/3B1/3B2 555
ENO1 ENO2 ENO3 661
BPGM PGAM1/2/4 1029
TPI1 1141
ME1 ME3 1535
GALM 1844
LDHA/B/C/AL6A/AL6B 1849
TKT TKTL2 2220
ACSS2 ACSS1 2832
PGLS H6PD 3780
GAPDH 3898
ADH 5835
MDH1 MDH2 6054
AKR1A1 6177
ACYP1 ACYP2 6366
G6PC 6508
FBP1 FBP2 6740
RPE 7138
TALDO1 7198
PRPS1 PRPS1L1 7891
DERA 8026
RPIA 9061
GOT1 GOT2 11728
PGD 11731
DLD 11818
GCK 12608
PGM1 PGM3 13299
BRE 17327
PDHA1 PDHA2 PDHB 19352
G6PD 19768
GPT GPT2 20243
DLAT 25966
ALDH1A3/1B1/2/3A1/3A2/7A1/9A1 27300
max rank 28783

FDG-uptake x10^-3 [cpm/cell]

FDG signature prediction score

correlation coefficient

A: Glycolysis core
B: Average rank in FDG-uptake gene list
C: FDG signature prediction score
D: Correlation coefficient
INTEGRATED GENOMIC DATASET OF
LOCALLY ADVANCED HUMAN
BREAST CANCER (N = 80)(Chin et al.)

DETERMINE FDG SIGNATURE SCORE
FOR EACH TUMOR

Low FDG Signature Score (n = 18)
High FDG Signature Score (n = 18)

FDG-PET vs. Basal
(r=0.53, p-value<0.001)

FDG-PET vs. Luminal
(r=0.40, p-value<0.001)

FDG-PET vs. ERBB2
(r=0.0001, N.S.)

Chromosome number

FDG signature score

Transitions per chromosome (mean)

FDG-PET

FDG PET

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 X Y 22 23

FDG uptake

SUV>10 SUV<5

0.5 1.0 1.5 2.0 2.5 3.0

HIGH LOW

FDG signature score

p=6x10^{-6}

p<0.05
A. Table showing gene sets and their associated NES, NOM, and p-values:

<table>
<thead>
<tr>
<th>Rank</th>
<th>Geneset name</th>
<th>NES</th>
<th>NOM</th>
<th>p-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>cancer_undifferentiated_metas_up</td>
<td>3.40</td>
<td>&lt;0.001</td>
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<tr>
<td>2</td>
<td>serum_fibroblast_cellcycle</td>
<td>2.88</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BT03 IHC positive</td>
<td>1.94</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>4</td>
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<td>1.70</td>
<td>0.002</td>
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<tr>
<td>5</td>
<td>BT06 IHC negative</td>
<td>-2.20</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>BT07 IHC negative</td>
<td>-1.81</td>
<td>0.012</td>
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<tr>
<td>7</td>
<td>BT08 IHC positive</td>
<td>-2.08</td>
<td>&lt;0.001</td>
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<tr>
<td>8</td>
<td>BT09 IHC positive</td>
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<td>&lt;0.001</td>
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<td>BT16 IHC positive</td>
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<td>&lt;0.001</td>
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<td>BT17 IHC positive</td>
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<td>&lt;0.001</td>
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<td>BT18 IHC positive</td>
<td>-4.46</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

B. IHC images showing MYC positivity and negativity.

C. Heatmap showing MYC expression levels.

D. FDG signature score p-values.
18F-fluorodeoxy-glucose positron emission tomography (18FDG-PET) marks MYC-overexpressing human basal-like breast cancers


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